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Monoblastic Leukemia in an HIV-Infected Patient: Absence of Viral Expression in RNA Blasts

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A small number of patients seropositive for the human immunodeficiency virus (HIV) have been reported as developing acute non-lymphoblastic leukemia (ANLL). In the cases previously published, the authors never reported a study of the link joining HIV infection and leukemia. We describe here the case of a 41-year-old HIV positive patient who developed ANLL (FAB classification M5). Using molecular techniques, we looked for a direct link between these two co-existing diseases. We showed the absence of HIV expression in the malignant clone, suggesting that the association of ANLL and Acquired Immune Deficiency Syndrome is not a direct consequence of the myeloid precursors infection. Nevertheless a relationship may exist through a disorganization of the bone marrow micro-environment. © 1996 Wiley-Liss, Inc.

Key words: acute nonlymphoblastic leukemia (ANLL), Southern and Northern blot

INTRODUCTION

In patients infected with the human immunodeficiency virus (HIV), hematological malignancies mainly consist of lymphomas of B-cell origin [1,2], but a few cases of acute nonlymphocytic leukemia (ANLL) have also been reported [3–9]. Except for two reported cases [6,9], ANLL patients have been unsuccessful in achieving remission. The finding of patients with the two coexisting diseases, acquired immunodeficiency syndrome (AIDS) and acute leukemia, raises the question of a possible link between HIV-1 infection and leukemogenesis. At the present time no study has investigated whether or not such a relationship exists.

In the present article we report on a 41-year-old HIV-positive man with ANLL in whom we looked for virus gene integration in ANLL blasts.

CASE REPORT

A 41-year-old HIV homosexual patient known to be HIV antibody positive (screened by enzyme-linked immunosorbent assay and confirmed by Western blot) since October 1989 was admitted to the Centre d'Information et de Surveillance de l'Immunodeficience Humaine (CISIH) in July 1990 (month 0, Fig. 1). He had a history of occasional infections including herpes and gonococcal infections and maxillar adenopathies, but his clinical and biological status was stable until January 1992, when his CD4 T-lymphocyte subsets fell to 0.292×10^9/l. This justified the inclusion of this patient in the Agence Nationale de Recherche sur le SIDA (ANRS) Clinical Trial Delta that randomizes zidovudine alone versus zidovudine and Dideoxyinosine (DDI) versus zidovudine and Dideoxycytidine (DDC). The patient received zidovudine with or without DDC. This treatment started in July 1992 (month 24), followed by stabilization of CD4 T-cell count at a level higher than 0.4×10^9/l. Regression of the adenopathies was observed. Biological and clinical parameters were remarkably stable until June 1993 (month 35), as shown by hematology follow-up (illustrated in Fig. 1). The first notable abnormalities were neutropenia increasing with time (August 1993: 1.435×10^9/l; October: 1.260×10^9/l; January 1994: 0.879×10^9/l) and repeated infections. In January 1994 a physical examination re-

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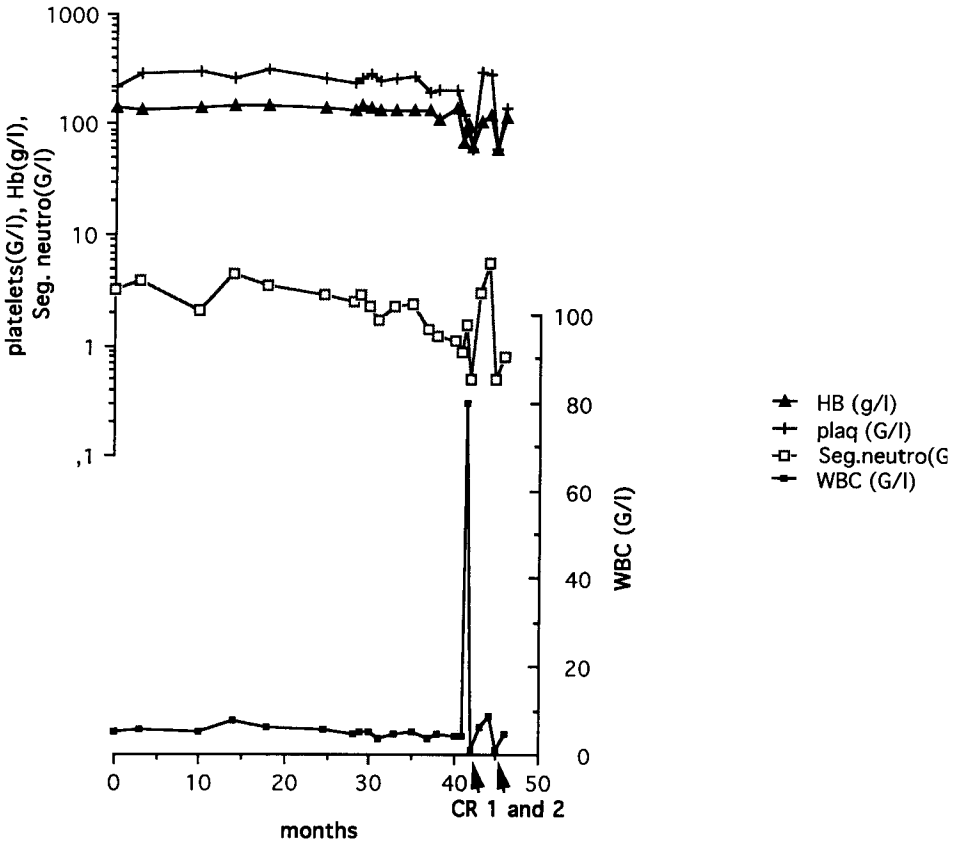

Fig. 1. Peripheral blood data during 46 months of monitoring from CISIH inclusion unt leukemia diagnosis (month 41.5) and first and second cytologic remissions (months 4 and 45, respectively). Changes in hemoglobin (g/l), platelet (G/l), leukocyte (G/l), an segmented neutrophil (G/l) counts before and after treatment for ANLL. CR, cytologic r mission.

vealed increased adenopathies, hepatosplenomegaly, gingival hypertrophy, and nodular cutaneous lesions of the back and the thigh. Another lesion of the foot suggested Kaposi's sarcoma. Blood count showed a hemoglobin of 102 g/l, white cell count of 80 × 10^9/l with a differential of 2% neutrophils, 7% lymphocytes, 4% monocytes, and 87% hemoblasts, red cell count of 2.75 × 10^{12}/l, and platelet count of 90 × 10^9/l. He was then hospitalized. At this time peripheral blood T-lymphocyte subsets were 0.608 × 10^9/l CD4 cells and 1.358 × 10^9/l CD8 cells (Fig. 2). Morphologically, peripheral blood blasts appeared to be monoblastic. Examination of skin biopsy from the thigh showed a blastic infiltration, and another biopsy from the foot confirmed the diagnosis of Kaposi's sarcoma. An attempt to culture the blasts for cytogenetic analysis was unsuccessful.

Induction chemotherapy was initiated with daunorubicin (DNR) and cytosine arabinoside (Ara C). This was complicated by pulmonary infection requiring broad-spectrum antibiotics. Twenty days after initiation of treatment a partial remission was obtained (hematologic remission according to bone marrow aspirate and blood

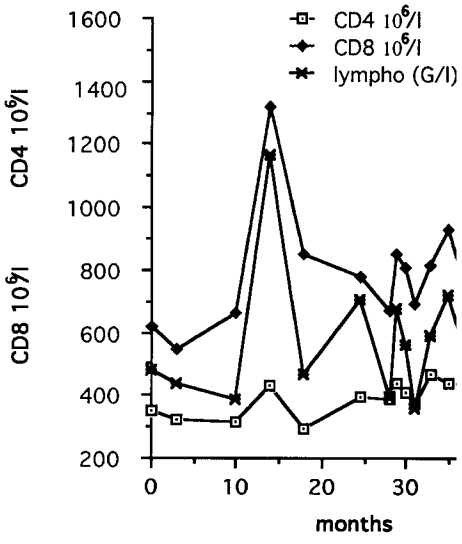

Fig. 2. Changes in lymphocytes (G/l) a subsets (10^6/l) before and after treatment

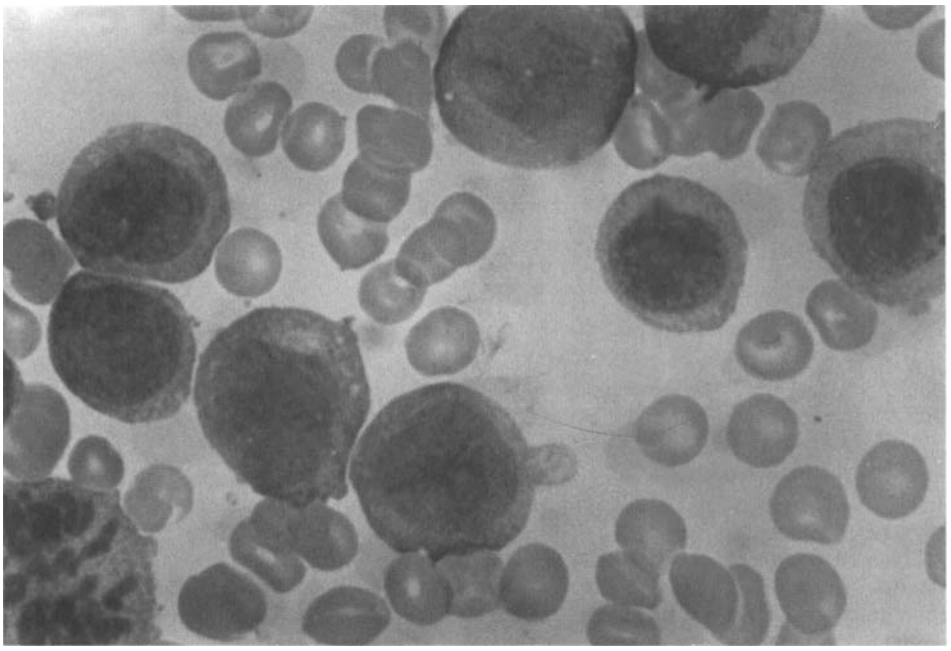

Fig. 3. Cells present in the bone marrow at time of leukemia diagnosis: leukemic blasts stained with May-Grunwald-Giemsa (×1,000).

samples), but the cutaneous lesions never totally disappeared. Two months later a blood relapse occurred (month 44). Another chemotherapy course with mitoxantrone, etoposide, and ciclosporine was started but only a temporary reduction of the peripheral blasts was obtained. The patient died in August 1994.

MATERIALS AND METHODS

Cytology

Differential counts of the bone marrow aspirate and peripheral blood were performed by the May-Grunwald-Giemsa staining technique. Cytochemical studies were performed on bone marrow aspirate using myeloperoxidase and an α-naphthyl butyrate esterase reaction.

Immunophenotyping and Flow Cytometric Analysis

The antibodies used were those recommended by the Groupe d'Etude Immunologique des Leucémies (GEIL): CD2 (S.T11), CD3 (S.T3), CD4 (S.T4), and CD5 (S.T1) purchased from Biosys (Compiègne, France); CD19 (B4; Coulter, Margency, France); and CD10 (IOB5a; Immunotech, Marseille, France) as lymphoid markers; CD13 (My7) and CD33 (My9) (Coulter); CD11b, CD14, DR (IOM1, IOM2, and IOT2a; Immunotech); and CD15 (S.My15a; Biosys) for the myelomonocytic lineage. Surface antigen expression measured by flow cytometry was done with an indirect staining technique. Briefly, cells were incubated with the first-step MAb under saturating concentration for 60 minutes at 4°C, washed twice in phosphate-buffered saline containing 1% bovine serum albumin and 0.1% azide, and then incubated for 45 minutes with the second-step fluorescein isothiocyanate-labeled goat antimouse antibody (Silenus, Eurobio, Paris). Flow cytometry assay was performed using an Epics Profile II instrument (Coulter).

Detection of Viral Expression in Blasts

Blast DNA and RNA were extracted from the initial sample containing 87% blasts. Highly sensitive molecular techniques such as polymerase chain reaction (PCR) were performed as previously described [10]. Southern and Northern blot techniques were also performed, as previously described [11] on the same sample. The H9 cell-line, an immortalized T-cell line expressing high levels of CD4, was used to propagate HIV virus in vitro; cells were provided by the American Type Culture Collection. The HIV molecular clone containing the complete genome of the HIV-1_{NDK} isolate was used as previously described [12]. DNA was digested with *Hin*dIII restriction enzyme, and the restriction fragments were hybridized with a plasmid containing a complete molecular clone of HIV-1 [13] labeled with phosphore 32.

RESULTS

Bone marrow aspirate revealed 94% blasts, also with a monoblastic appearance (Fig. 3). Cytochemistry showed

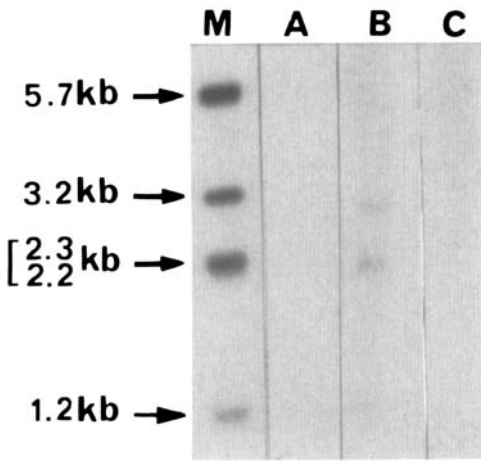

Fig. 4. Southern blot hybridization of *Hin*dIII restriction fragments of the DNA corresponding to lane (A) uninfected H9 cells, lane (B) HIV-1-infected H9 cells, and lane (C) patient sample. M is a molecular weight marker. After electrophoresis on a 0.8% agarose gel, fragments were transfered onto a nylon membrane (Gene Screen Plus, Dupont) and hybridized with the HIV-1 NDK probe labeled with ^{32}P according to the manufacturer's instructions.

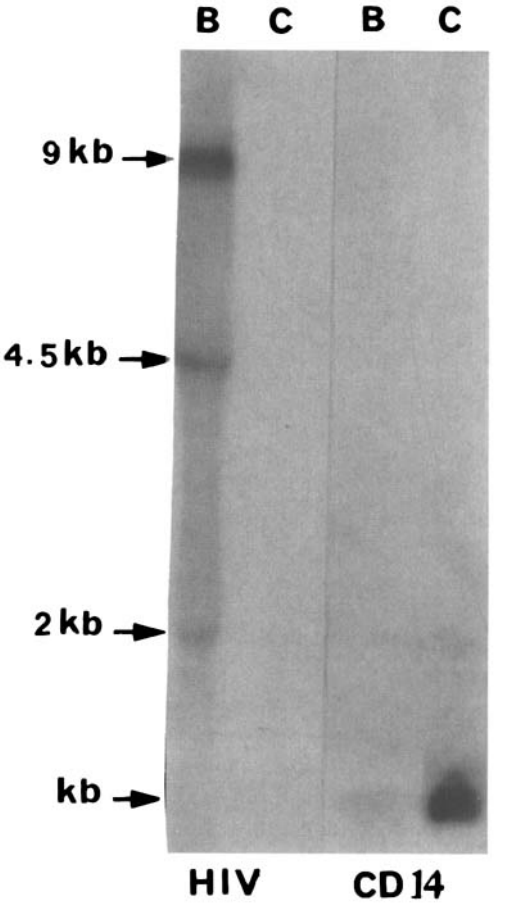

Fig. 5. Northern blot hybridization of RNA corresponding to (lane B) HIV-1-infected H9 cells, and (lane C) patient sample. After electrophoresis on a 1% agarose gel, the fragments were transferred onto a nylon membrane (Gene Screen Plus, Dupont) and hybridized either with the HIV-1 NDK probe labeled with ^{32}P (left side) or with the CD14 probe labeled with ^{32}P (right side) according to the manufacturer's instructions.

a low reaction for peroxidase for 55% of blasts and a high positivity with α-naphthyl butyrate esterase for all blasts. Flow cytometry analysis revealed that blast cells were positive for CD13, CD33, CD11b, CD14, DR, and CD15. Morphology, cytochemistry, and immunology were those of FAB-M5 [13,14] (monoblastic) ANLL.

Viral sequences using primers matching two HIV conserved regions (nucleotides 5032–5053 and 5605–5584) were detected by PCR (data not shown). However, this positivity is likely due to the presence of contaminating CD4+ cells in the sample. To determine whether the tumor cells were infected, molecular analyses were performed using Southern and Northern blot techniques [11]. These methods are less sensitive than PCR and can detect HIV signals in an infected homogeneous cellular population. No hybrid fragment was detected in blast DNA, suggesting the absence of cloned provirus integration in the malignant clone (Fig. 4). Furthermore, total cellular RNA extracted from the same sample were analyzed by the Northern blot technique. The results (indicated in Fig. 5) revealed the three types (unspliced, monospliced, and multispliced) of HIV transcripts in infected CD4+ cells (H9 cells); however, no signal was observed in the tumor RNA. The integrity of the tumor RNA was checked by hybridization with a CD14 probe [15], which revealed a strong 0.8 kb transcript. The CD14 probe corresponds to an *SstI-XhoI* cDNA fragment kindly provided by Dr. S. Goyert (North Shore University Hospital).

DISCUSSION

Hematologic abnormalities are commonly associated with HIV infection. Bone marrow examinations reveal frequent dysplasia, dyserythropoiesis, lymphocytic infiltration, and plasmocytosis [16–20]. These marrow abnormalities are strongly associated with peripheral blood cytopenia of one or more lineages such as anemia, granulocytopenia, or thrombocytopenia, which are frequently described [22–23]. The pathogenic mechanisms of these changes are complex. Indeed, the virus itself can be directly or indirectly responsible. Moreover, antiretroviral or antimicrobial treatments, which are frequently employed, are not devoid of hematotoxicity. Understanding the real causes of impaired hematopoiesis in HIV-infected patients is therefore difficult. In 1985, Schneider and Picker reported myelodysplasia similar to that of the myelodysplastic or preleukemic syndroms in eight patients

with AIDS [24]. They suggested that the dysplasia may contribute to peripheral cytopenia through ineffective haematopoiesis but pointed out that no patients with AIDS has ever been reported with acute leukemia. Since that time, eight cases of ANLL have been reported to develop in AIDS [3–9], but no study has clearly demonstrated an increased risk of ANLL in HIV infection. Thus the significance of the association between HIV infection and myeloblastic leukemia must be viewed with caution.

It is well known that CD4 expression by cells of the myelomonocytic lineage make them a target for HIV, and recently low levels of CD4 expression were detected in bone marrow early progenitors [25]. Numerous well-documented studies have shown the presence of the HIV in CD4-positive myelomonocytic cells in well-differentiated stages such as blood monocytes [26,27] or brain macrophages [28], as well as in more immature mononucleated cells. Indeed, in situ hybridization with cDNA probes on bone marrow tissue sections from AIDS and AIDS-related complex patients showed the presence of HIV nucleic acids in myelocytes and myeloblasts [29]. The detection of HIV RNA in myeloid precursors from HIV-seropositive individuals was also reported [30]. Thus the monocytic lineage can be considered a real sanctuary for HIV and highly involved in virus replication. Investigations of possible causes of leukemogenesis in AIDS appear justified, especially experiments attempting to demonstrate a direct infection of blast cells by the virus, which has never been done before.

In the case reported here, using molecular biology techniques and specific virus DNA probes, we failed to detect hybrid fragments in DNA monocytic blasts from an HIV antibody-positive patient. The absence of viral expression, together with the lack of CD4 detection in the malignant cells, allowed us to conclude that there was no direct link between HIV infection and acute monocytic leukemia. Nevertheless, an indirect connection may exist through the profound cell immunodeficiency of AIDS, possibly due to the defective T-cell control of hematopoiesis or a failure of immune surveillance. There have been reports of the consequences of HIV infection on bone marrow stroma [31], specifically on histiocytic and endothelial cells, which may disturb cytokine production. The resulting hematopoiesis dysregulation may be involved in the leukemogenesis mechanism. In this case, the absence of HIV integration in blasts constitutes a strong argument that HIV per se is not a direct cause of ANLL. However, it would be careless to dismiss the possibility of any relationship between the two diseases. Indeed, an indirect link may exist between HIV infection and leukemogenesis through disorganization of the bone marrow microenvironment caused by stroma cell infection and hematopoietic dysregulation.

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